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## **DIVISION S-7–NOTES**

# INORGANIC NITROGEN DETERMINED BY LABORATORY AND FIELD EXTRACTIONS OF TWO FOREST SOILS

HELGA VAN MIEGROET\*

#### Abstract

To assess the effect of a delay in soil processing on inorganic N levels in N-rich soils, field and laboratory extractions were compared at two forested sites with high N mineralization and nitrification potential. At eight sampling dates in 1989 and 1990, five mineral soil cores per site were taken between 0- and 10-cm depth and transported on ice to the laboratory for KCl extraction and NH<sub>4</sub>-N and NO<sub>3</sub>-N analysis. At three sampling dates in 1990, soil extractions were performed in the field immediately following sampling, and inorganic N concentrations were compared between extractions. Nitrate-N increased four- to sevenfold (net release of 2-7 mg NO3-N/kg dry soil) due to the transport and relatively short delay (<24 h) in the processing of the soil samples, either coinciding with increased net N mineralization or due to transformation of NH4-N into NO3-N. This study indicates that if possible, soil samples should be extracted in the field, especially at N-rich sites. The concerns raised by this study may not necessarily apply to N-poor soils characterized by slow N transformation rates.

ONE OF THE PROBLEMS that still exists in soils research is our inability to accurately quantify N transformations that take place in the soil under field conditions. Field characterization of soil N dynamics is important not only in terms of N availability to plants but also the characterization of N retention capacity (and conversely N saturation) and NO<sub>3</sub> leaching potential of forest ecosys-

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tems. The latter is especially relevant in areas impacted by high N inputs from the atmosphere. It is generally recognized that various laboratory incubations provide at best an index of N availability and N transformations that may occur under field conditions (e.g., Binkley and Hart, 1989). On the other hand, even when field methods are used (e.g., buried bags or resin cores), anomalous readings may result from soil disturbance. It is, for example, not uncommon to observe surges in nitrification following lysimeter installation (e.g., Johnson et al., 1991). Stark (1991) found that disturbance of oak woodland soils in California increased ammonification fivefold and doubled microbial NH4 consumption, but did not significantly increase nitrification. Because of the potential influence of biological transformations on the amount and the form of inorganic N in the soil, Keeney and Nelson (1982) suggested that samples taken for the determination of inorganic N forms should ideally be analyzed immediately after sampling to obtain valid results. Longterm storage and manipulation of soils (e.g., drying) between sampling in the field and N extraction in the laboratory has been shown to influence chemical analysis results (Edmeades et al., 1985; Ross et al., 1989; Ross and Bartlett, 1990). There is evidence that even storing field-moist soils in the refrigerator for short time periods (ranging from 24 h to 4 d) may elevate NO<sub>3</sub> levels in the extraction solution (Edmeades et al., 1985; Ross and Bartlett, 1990).

These observations raise the fundamental question whether the procedures commonly used during field incubations (extractions of pre- and postincubation soil cores in the laboratory) may bias results in some instances, even when precautions are taken to avoid warming of the soils during transport and efforts are made to minimize the time between soil sampling and processing. For example, soil solutions obtained through centrifugation from two high-elevation soils in the southern Appalachians showed unusually high NO<sub>3</sub> levels and raised

Department of Forest Resources, Utah State Univ., Logan, UT 84322-5215. Received 10 Jan. 1994. \*Corresponding author (helgavm@cc. <sup>Usu.edu)</sup>.

concerns about possible artifacts associated with sample processing (Van Miegroet et al., 1990). A small study was therefore initiated at two forested high-elevation sites in the Smoky Mountains to investigate the nature and extent of changes in the inorganic N composition of soil samples between the time of sampling in the field and the time soil samples are normally processed in the laboratory. Because the inherent characteristics of the soils in question suggested high net mineralization and nitrification capacity (e.g., high total soil N content, low C/N ratio, and high NO<sub>3</sub> leaching) (Van Miegroet et al., 1992), differences in inorganic soil N content between field and laboratory extractions, if observed, were expected to represent a worst-case scenario in terms of potential N mineralization response associated with the manipulation and storage of soil cores prior to processing.

### **Materials and Methods**

A comparison between field and laboratory extractions was conducted at two northeast-facing sites at 1950- and 1720-m elevation in the Great Smoky Mountains National Park near Clingman's Dome, North Carolina. A more detailed description of the sites can be found in McLaughlin et al. (1990) and Van Miegroet et al. (1993). The soils underlying the sites were derived from Thunderhead sandstone (National Park Service, 1984), are classified as Umbric Dystrochrepts or Typic Haplumbrepts, and have silt loam to sandy loam texture. The soils at the upper site have a thicker Oe horizon and generally higher organic matter content.

During the 1989 and 1990 growing seasons, five soil cores were taken with a bulb planter to a depth of  $\approx 10$  cm in each site after the O horizon had been cleared from the sampling point. The sampling dates were: June, July, and September 1989 and May, June, August, September, and October 1990. Sampling points were in close proximity to tension lysimeters that were part of a soil solution characterization study (Van Miegroet et al., 1990). Soil samples were immediately placed in plastic bags after collection. At three sampling dates (May, June, and October 1990), subsamples (10-20 g fresh weight) were removed from the bag in the field and added immediately to a preweighed plastic bottle containing 100 mL of 2 M KCl. These samples represent field extractions. Bottles with soil-KCl mixtures and the soil bags were transported on ice to the laboratory, and placed in the refrigerator on arrival. All samples were processed within 24 h of arrival in the laboratory. At the different sampling dates, soil temperatures at the 5-cm depth were also recorded at each sampling point using moisturetemperature cells that had been installed at the onset of the study (Soiltest, Evanston, IL).

In the laboratory, field extraction bottles were reweighed to determine the exact amount of field-moist soil added. Laboratory soil extractions were done by adding 100 mL of 2 *M* KCl to 10 g of fresh soil. Two blanks (KCl only) were consistently included in each extraction batch to determine procedural contamination. All soil-KCl mixtures were shaken for  $\approx 1$  h and allowed to settle for 1 h, after which the supernatant was filtered (Whatman no. 42) and the filtrate analyzed for NH<sub>4</sub>-N and NO<sub>3</sub>-N on an autoanalyzer (Technicon Instrument Corp., Tarrytown, NY). Moisture content was determined gravimetrically on subsamples (10–20 g) oven dried for at least 24 h at 105°C and cooled in a desiccator. All N concentrations are expressed on an oven-dry-weight basis. Differences in the contact time between soil samples and the extractant between the field and laboratory extractions and its potential influence on extractable soil N concentrations were not explicitly considered in this study. Although it is not possible at this point to preclude with certainty any effect of contact time on the amount and the form of N in the extractant, there seems to be little evidence in the literature to substantiate or quantify such influence, particularly with regard to 2 M KCl extractions and the processing times used in this study. Indeed, Keeney and Nelson (1982) suggested that little change in the extractable inorganic N values should be expected if soil-KCl suspensions are left unfiltered for up to 24 h. Further, published methods for soil analyses seem to suggest that KCl extractions yield good inorganic N recovery, are relatively stable, and are reliable even when shaking times are varied (Bremner, 1965).

Variations in laboratory-extractable inorganic N forms due to sampling time were tested for each site using a one-way analysis of variance, and significant differences between means were determined by the least significant difference test (P =0.05). Differences in extractable inorganic N content between field and laboratory extractions were tested with a paired *t*-test for the upper and lower sites at different sampling dates (n =5 per site and sampling date). The influence of physical soil properties (temperature and moisture content) on variability in extractable N was determined by stepwise regression analysis (SAS Institute, 1985).

### **Results and Discussion**

At both sites, extractable inorganic N concentrations in the mineral soil obtained through conventional laboratory extractions varied considerably with time, ranged from <5 to 33 mg/kg, and were highest in 1989 (Table 1). The NO<sub>3</sub>-N concentrations at the upper site varied between 3 and 19 mg/kg and followed a similar temporal pattern, i.e., higher concentrations were generally measured in 1989, concentrations were generally lowest in the summer of 1990, and concentrations tended to increase again toward the fall of 1990. The average NO<sub>3</sub>-N levels at the lower site varied somewhat less between sampling dates and were mostly on the order of 5 to 10 mg/kg. Overall, NO3-N accounted for 30 to 100% of total laboratory-extractable mineral N. Such a large contribution of NO3-N in the inorganic N pool is consistent with other observations in high-elevation spruce-fir forests in the southeastern U.S.A. (Joslin et al., 1992), and confirms the high N availability and nitrification potential at these sites indicated in earlier studies (Johnson et al., 1991; Van Miegroet et al., 1992).

The temporal and spatial fluctuations in extractable inorganic N and NO<sub>3</sub>-N across all mineral soil samples (n = 80) were significantly influenced by soil moisture content, which explained 22 and 24% of the variation (P < 0.0001) in total inorganic N and NO<sub>3</sub>-N concentrations, respectively. The relatively dry conditions in the spring and summer of 1990 accounted for the generally lower inorganic N levels at both sites, whereas the higher (more normal) moisture relations in the previous year were associated with higher inorganic N levels in the mineral soils of both sites (Table 1). The same relationship also held when samples from the upper and lower site

Date	Temperature	Moisture	Laboratory extractions <sup>†</sup>			Laboratory/field extractions†		
			NH4-N	NO <sub>3</sub> -N	Inorganic N	NH₄-N	NO <sub>3</sub> -N	Inorganic N
-	°C	% dry soil		mg/kg				14
				U	pper site			
June 1989 July 1989	$\begin{array}{c} 8.6\ \pm\ 1.4\ddagger\\ 12.8\ \pm\ 1.0 \end{array}$	$\begin{array}{r} 159 \ \pm \ 49 \\ 103 \ \pm \ 50 \end{array}$	$\begin{array}{r} 4.1\ \pm\ 2.4\ cd\$\\ 12.2\ \pm\ 5.8\ b\end{array}$	$\frac{18.7 \pm 6.3 \text{ a}}{12.5 \pm 4.2 \text{ bc}}$	$\begin{array}{c} \textbf{22.8} \ \pm \ \textbf{8.4} \ \textbf{b} \\ \textbf{24.7} \ \pm \ \textbf{9.2} \ \textbf{b} \end{array}$			
Sept. 1989 May 1990	$\begin{array}{c} 12.7 \pm 1.0 \\ 7.3 \pm 0.6 \\ 12.7 \pm 1.0 \end{array}$	$160 \pm 53$ 72 ± 23	$24.5 \pm 9.0 \text{ a}$ 1.7 ± 1.3 cd	$9.0 \pm 1.8 \text{ cd}$ $4.5 \pm 3.3 \text{ de}$	$33.5 \pm 9.1 a$ 6.2 ± 4.6 d	$0.4 \pm 0.3$	$4.9 \pm 3.4$	$1.1 \pm 0.3$
June 1990 Aug. 1990	$12.7 \pm 1.0$ $12.6 \pm 1.4$ $9.8 \pm 0.8$	$102 \pm 12$ 107 ± 38 110 + 16	$1.8 \pm 0.7 \text{ cd}$ $2.8 \pm 1.4 \text{ cd}$	$4.4 \pm 1.3 \text{ de}$ 3.3 ± 1.9 e 14.6 + 7.0 ab	$4.4 \pm 1.5 d$ 5.1 ± 2.2 d 17.4 + 7.9 bc	$0.0 \pm 0.0$	2.8 ± 0.2	$0.8 \pm 0.2$
Oct. 1990	$7.6 \pm 0.3$	$118 \pm 44$	$6.8 \pm 2.7 c$	$4.6 \pm 0.5$ de	$11.3 \pm 2.7 \text{ cd}$	$0.5~\pm~0.2$	$4.5 \pm 1.6$	$0.7~\pm~0.3$
Plot mean						$0.5 \pm 0.2 \ (n = 10)$	$4.2 \pm 2.3 \ (n = 14)$	$0.9 \pm 0.3 (n = 15)$
				Le	ower site			
June 1989 July 1989	$\begin{array}{c} 12.2 \pm 1.7 \\ 13.6 \pm 0.6 \\ 13.3 \pm 0.4 \end{array}$	$198 \pm 104$ $108 \pm 111$ $149 \pm 80$	$3.5 \pm 0.9$ bc 16.5 $\pm 23.2$ a 14.2 $\pm 4.0$ ab	$9.9 \pm 3.7 \text{ abc}$ 11.2 ± 6.7 ab 7.9 ± 5.7 bc	$13.4 \pm 4.5$ bc 27.7 ± 25.1 a 22.1 ± 7.0 ab			
Sept. 1989 May 1990 June 1990	$6.9 \pm 0.3$ 11.9 + 0.7	$49 \pm 14$ 127 + 61	$1.9 \pm 1.7 \text{ c}$ 1.2 + 2.5  c	$2.7 \pm 1.3 \text{ c}$ $7.7 \pm 2.9 \text{ bc}$	$4.6 \pm 1.2 \text{ c}$ $8.9 \pm 5.2 \text{ bc}$	$8.9 \pm 15.9 (n = 4)$ $0.2 \pm 0.3$	$7.7 \pm 8.3$ 5.0 + 5.0	$4.3 \pm 3.5$ $1.6 \pm 0.6$
Aug. 1990 Sept. 1990	$12.3 \pm 0.5$ 10.6 ± 0.6	$     \begin{array}{r} - \\       84 \pm 22 \\       97 \pm 18     \end{array} $	$2.2 \pm 0.8 c$ $0.0 \pm 0.0 c$	$6.3 \pm 3.8 \text{ bc}$ 16.8 ± 8.0 a	$8.6 \pm 4.1 c$ 16.8 ± 8.0 abc			
Oct. 1990	$9.9~\pm~0.3$	$100 \pm 24$	$4.6~\pm~0.7~bc$	$8.4 \pm 7.7 \ bc$	$12.9 \pm 8.3 \text{ bc}$	$0.5 \pm 0.1$	$7.8 \pm 6.8$	$1.4 \pm 1.4$
Plot mean						$3.5 \pm 9.7 (n = 11)$	$6.8 \pm 6.4 \ (n = 15)$	$2.4 \pm 2.4 \ (n = 15)$
Overall mean			1.			$2.1 \pm 7.0 \ (n = 21)$	$5.5 \pm 5.0 \ (n = 29)$	$1.6 \pm 1.9 \ (n = 30)$

Table 1. Average temperature, moisture content, and inorganic N concentration of soils determined by laboratory extraction sampled at different dates, and the relationship between field- and laboratory-extractable inorganic N in soils from two high-elevation sites in the Great Smoky Mountains.

 $t_n = 5$  except where indicated otherwise.

t Mean ± standard deviation.

S Different letters following mean inorganic N concentrations indicate statistically significant differences among sampling dates at a given site (P < 0.05).

were analyzed separately: the  $r^2$  between soil moisture content and mineral N in the soil was 0.20 (P < 0.01) at the lower site and 0.28 (P < 0.001) at the upper site; the  $r^2$  between soil moisture and NO<sub>3</sub>-N was 0.28 (P < 0.001) at the lower site and 0.21 (P < 0.01) at the upper site. Temperature was not a statistically important environmental factor contributing to variations in inorganic soil N. Although the soils at the lower site tended to be somewhat warmer, these differences had no marked effect on the amount of extractable soil N at the two sites. The results from the regressions analysis (i.e., at most one-fourth of variability explained) suggests that site and environmental factors other than those considered in this analysis appear to control variations in inorganic soil N concentrations.

There were considerable differences in the inorganic N fraction between field and laboratory extractions (Fig. 1). With a delay in soil processing, an increase in  $NO_3$ -N of 2 to 7 mg/kg was measured that was statistically significant in most instances. This represented on average a fourfold increase in soil NO<sub>3</sub>-N concentrations between field and laboratory extraction at the upper site and a sevenfold increase at the lower site. In almost all instances there was a concurrent decline in extractable NH<sub>4</sub>-N that was largest in October 1990, and generally more pronounced in the upper site. In four out of six cases, differences in NH<sub>4</sub>-N between field and laboratory extractions were statistically significant (Fig. 1).

Because of the longer contact time between the soil and the extractant in the field extractions, there may be some concern that the two extractions are not truly comparable, and especially that unaccounted changes in inorganic N levels may have been caused by transformations in the soil-KCl mixtures within the period ( $\leq 24$  h) prior to filtration of the field extracts. As also indicated above, there is currently little or no hard evidence in the literature that substantiates significant changes in the chemical composition of forest soil-2 M KCl mixtures within a period of 24 h due to chemical or enzymatic reactions. Keeney and Nelson (1982) recommend filtration of soil-KCl suspensions and cool storage of the filtrates (rather than the suspensions) if analysis cannot be performed soon (within 24 h) after extraction, because storage of unfiltered suspensions may affect the chemical analysis results. However, their recommendation implies



Fig. 1. Relative change in NH<sub>4</sub>-N and NO<sub>3</sub>-N concentrations between field and laboratory extractions of soil samples from two highelevation sites in the Great Smoky Mountains. Error bar indicates standard error about the mean. \*\*\*, \*\*, \* Significantly different from zero at the 0.001, 0.01, and 0.05 probability levels, respectively.

that when the lapse time between preparation and filtration of the soil-KCl suspensions is kept to  $\leq 24$  h, no significant changes in chemical composition should be expected. It was not possible in this study to ascertain to what extent biological activity occurred in the soil-KCl mixtures within a 24-h period, and to what extent it altered inorganic N levels. However, if biological activity had a significant influence on soil N chemistry, it would more likely have resulted in a net release of inorganic N rather than in microbial immobilization and net loss of inorganic N, considering the characteristics of the soil (high N content, low C/N ratio, high nitrification potential [Van Miegroet et al., 1992]). In other words, it would probably have resulted in an overestimate rather than an underestimate of initial field N levels. Further, even if chemical N transformations took place in the salt solutions, there is no reason to suggest that they would be higher than those taking place in the stored soil samples. The potential error associated with the longer soil-extractant contact time should therefore be relatively small compared with the magnitude and direction of the changes occurring in the fresh soils during that same period. However, the issue of potential biological N transformations in soil-KCl suspensions should be considered or investigated, especially in those cases where field logistics require storage of suspensions for longer than 24 h.

At the lower site, the net release of soil NO<sub>3</sub>-N between field and laboratory extractions increased between May and October and consistently exceeded the decline in NH<sub>4</sub>-N levels. In other words, there was an overall net increase in total inorganic N content of the soils from the lower site, not just a transformation of NH<sub>4</sub>-N into NO<sub>3</sub>-N. This net mineralization was also expressed by the ratio of laboratory-extracted to field-extracted N of  $2.4 \pm 2.4$  (n = 15) (Table 1). These results suggest that transport and storage of the soil samples originating from the lower site stimulated both net N mineralization and net nitrification, more than doubling the inorganic N content of the soil within a 24-h period. The results were somewhat different at the upper site in that the net release of NO<sub>3</sub>-N showed less variability in time and space and fluctuated around 3 mg/kg, while the apparent disappearance of NH<sub>4</sub>-N between field and laboratory extractions became larger from the May to the October assay. Overall, the total inorganic N content was more stable with sample manipulation at this site, indicated by a ratio of laboratory-extractable to field-extractable inorganic N content of nearly 1 (0.9  $\pm$  0.3 [n = 15]). Thus, in the samples originating from the upper site, the delay in soil processing did not cause a net release of inorganic N through N mineralization, only a change in the relative contribution of NH<sub>4</sub>-N vs. NO<sub>3</sub>-N. That is, it only stimulated net nitrification of NH<sub>4</sub>-N that was already present in the soil. In all cases, measurable net NO<sub>3</sub>-N release was consistently observed during transport of forest soil samples from the field and shortterm storage in the laboratory, and net nitrification rates were almost always higher than net N mineralization of soil organic N, irrespective of the site from which the forest soil samples were taken.

Because the comparisons happened to coincide with (drier) periods when extractable N levels were inherently low (May, June, and October 1990), it is not possible to quantify the exact rate of N release between soil sampling and processing during periods when the soils were wetter and characterized by higher N activity (e.g., 1989). However, there is no apparent reason to suggest that the absolute NO<sub>3</sub>-N release rates during transport and short-term storage of the 1989 soils would have been any lower than those measured in the 1990 samples. especially in view of the positive correlation between soil moisture content and soil NO3-N levels. Consequently, the discrepancies in inorganic N concentrations between field and laboratory extractions in this study may, in fact, not represent a worst-case scenario for these forest soils.

## Conclusions

This comparative study has shown that in soils that have inherently high mineralization and nitrification potentials, transportation from the field and short-term storage of soil samples may stimulate turnover of soil organic N, and especially oxidation of NH<sub>4</sub>-N, even if precautions are taken to minimize processing time and avoid warming of the soil samples until processing. Conventional laboratory extractions tended to consistently overestimate NO<sub>3</sub>-N levels in these soils by as much as fivefold. The effect on soil NH<sub>4</sub>-N and total inorganic N levels was less consistent: increases, decreases, or no changes in inorganic soil N levels were observed, depending on the position in the landscape and time of sampling. In some instances, only acceleration of net nitrification of inherent NH4-N was observed, whereas in others the delay in processing of these N-rich soils stimulated both net mineralization of organic soil N and further nitrification and release of NO<sub>3</sub>-N.

The observed changes in the concentration and composition of inorganic soil N may have implications for characterization of soil N status and N cycling patterns, as well as for the evaluation of the relative abundance of the different N forms available for plant uptake. Because the observations in this study were derived from only two sites, known to have high N turnover rates, the patterns described here may not apply to all forest soils, and especially not those that are N poor and are characterized by inherently low N transformation rates. However, this study has illustrated the potential limitations of using laboratory procedures to describe the N status under field conditions. Also, with the increasing emphasis placed on the importance of atmospheric N inputs to the degree of N saturation (i.e., the extent of N retention within the ecosystem), expressed by the rate and periodicity of NO3-N production and leaching (Aber et al., 1989), it will become more critical to provide measures of net field nitrification with some degree of accuracy. It is therefore recommended that soil samples, especially those with known high N mineralization and nitrification potential, be processed in the field to the extent possible if the objective of the assays is to reflect field conditions as closely as possible.

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